V12

Ascorbates

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SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the investigation of ERYTHORBIC ACID in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of
GRAS Substances to the Developing Chicken Embryo:

ERYTHORBIC ACID

PROTOCOL:

Erythorbic acid (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered, with water as the diluent, by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2, 3).

Groups of ten or more eggs were treated under these four conditions at several dose levels until a suitable total number of eggs per level was reached for all levels allowing some to hatch. Groups of adequate size were treated solely with the solvent at corresponding volumes. Untreated controls were also included in each experiment.

After treatment, all the eggs were candled daily and the non-viable embryos were removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. Along with these, histological examinations of major organs (liver, heart, kidney, lung, brain, intestine, gonad, and some endocrine organs) were carried out by taking samples from a representative number of animals from each experimental group. All abnormalities were tabulated.

RESULTS:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram egg weight, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., the total number of non-viable eggs divided by the total number of treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total number of eggs treated. This includes all the abnormalities observed and also the toxic responses such as edema,

hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, and neural disorders including ataxia.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as a percentage of the total number of eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Columns 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data in columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

DISCUSSION:

Erythorbic acid was found to be quite embryotoxic when administered to the embryos under all conditions of the test. The toxicity was significantly ($P=0.05$) greater than solvent-treated eggs at all dose levels tested except 0.5 mg/egg via the air cell at 0 hours. Probit analysis resulted in an LC_{50} of 3.727 mg/egg (air cell at 0 hours, Table 1) and an LC_{50} of 4.472 mg/egg (air cell at 96 hours, Table 2). Yolk treatment at 0 hours resulted in an LC_{50} of 4.623 mg/egg (Table 3) and that at 96 hours of 5.426 mg/egg (Table 4).

Abnormal birds were seen under all four conditions of the test, but the incidence of birds having a structural abnormality of the head, limbs, viscera, or skeleton was not significantly different from the solvent background ($P=0.05$). Among a total of 180 untreated control eggs, only three abnormal birds, all having curled toes, were found.

AIR CELL AT 0 HOURS: Abnormalities were found in all of the dose levels without significant difference in frequency from the solvent-treated group, which had four abnormal birds (two with hip contracture and two with curled toes). At 20 mg/egg, three abnormal birds were found; two had celosomia and the other had curled toes. At 10 mg/egg, one bird had curled toes. At 5 mg/egg, two abnormal birds with hip contracture were found. At 1.0 mg/egg, five abnormal birds were found; three had hip

contracture and the remaining three had either curled toes or celosomia. At 0.5 mg/egg, only one bird was found to have celosomia.

AIR CELL AT 96 HOURS: All dose levels exhibited abnormalities; none was significantly different in frequency of abnormalities from that of the solvent-treated birds. At 20 mg/egg, three abnormal birds were found, one had abnormal maxillary curvature, the second bird had hip contracture and the third had hyperplastic eye. At 10 mg/egg, only one abnormal bird was seen with hip contracture. At 5.0 mg/egg, a bird with celosomia was the only abnormality found. At 1.0 mg/egg four abnormal birds were found, each with either hip contracture or toe contracture. The solvent-treated controls showed four abnormal birds, three had curled toes and one had celosomia.

YOLK AT 0 HOURS: Abnormalities were seen at all tested dose levels, including the solvent-treated controls. None of the levels, however, were found to be significantly different in frequency from the latter group. At 20 mg/egg, only one bird with curled toes was found. At 10 mg/egg, a total of four birds were abnormal; one with celosomia and the rest with curled toes. At 5.0 mg/egg, four abnormal birds were seen; three had curled toes and the fourth had hip contracture. At 1.0 mg/egg, three abnormal birds were found with either hip contracture or curled toes. The solvent-treated group had four abnormal birds, all with curled toes.

YOLK AT 96 HOURS: All of the tested levels showed abnormalities but the frequency of abnormalities was not significantly different from that of the solvent-treated birds. At 20 mg/egg, only one bird was abnormal with curled toes. At 10 mg/egg, four abnormal birds were seen; three had curled toes and the fourth had celosomia. At 5.0 mg/egg, two showed celosomia, two had curled toes and the fifth had hip contracture. At 0.1 mg/egg, two had curled toes and the third had hip contracture. The solvent-treated birds exhibited a total of eight abnormalities; seven had curled toes and the eighth had hip contracture.

From the above observations, the teratogenicity of erythorbic acid cannot be ascertained. None of the dose levels tested showed a percentage of abnormality significantly different from that of the solvent control. Most of the abnormalities described above were of the lower extremity and they were of the same varieties that were seen with the control animals.

Histological examinations of the major organs revealed no evidence of consistent change due to either the dose level of the substance administered or the mode of the treatment.

1. Erythorbic Acid (F.C.C., Fine granular), Lot #135102, FDA 3167 73 (C), Roche Chemical Division, Hoffman-LaRoche Inc., Nutley, N.J. 07110, FDA 71-66

2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M.J., Mutchler, M.K. and Fitzhugh, O.G. Toxicol. Appl. Pharmacol. 5:760-770, 1963

3. Verrett, M.J., Marliac, J.-P. and McLaughlin, J., Jr. JAOAC 47: 1002-1006, 1964

4. Finney, D.J. Probit Analysis, 2nd ed., Cambridge Press, Cambridge, Appendix I, 1964

Table 1

Erythorbic Acid

Air Cell at 0 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
20.0	400	122	88.52*	4.09	2.45
10.0	200	121	70.24*	1.65	0.82
5.0	100	124	56.45*	1.61	1.61
1.0	20	125	47.20*	4.80	4.00
0.5	10	56	17.85	1.78	1.78
Water		125	18.40	3.20	3.20
Control		180	11.11	1.66	1.66

LC_{30} 1.194 mg/egg (23.880 mg/kg)

LC_{50} 3.727 mg/egg (74.542 mg/kg)

LC_{90} 60.192 mg/egg (1203.855 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 2

Erythorbic Acid

Air Cell at 96 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
20	400	119	85.71*	2.52	2.52
10	200	68	69.11*	1.47	1.47
5.0	100	67	58.20*	1.49	1.49
1.0	20	67	56.71*	5.97	5.97
Water		119	21.00	3.36	3.36
Control		180	11.11	1.66	1.66

LC_{30} 1.370 mg/egg (27.407 mg/kg)

LC_{50} 4.472 mg/egg (89.456 mg/kg)

LC_{90} 80.560 mg/egg (1611.207 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 3

Erythorbic Acid

Yolk at 0 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
20	400	133	84.21*	0.75	0.75
10	200	133	73.68*	3.00	3.00
5.0	100	131	55.72*	3.81	3.05
1.0	20	131	54.96*	3.05	2.29
Water		196	25.00	2.04	2.04
Control		180	11.11	1.66	1.66

LC_{30} 1.260 mg/egg (25.220 mg/kg)

LC_{50} 4.623 mg/egg (92.479 mg/kg)

LC_{90} 110.901 mg/egg (2218.020 mg/kg)

*Significantly different from solvent $P \leq 0.05$

Table 4

Erythorbic Acid

Yolk at 96 Hours

Dose		Number	Percent	Percent Abnormal	
mg/egg	mg/kg	of eggs	Mortality	Total	Structural
20	400	139	82.73*	1.43	1.43
10	200	139	69.78*	3.59	2.87
5.0	100	139	58.99*	3.59	3.59
1.0	20	125	48.80*	2.40	2.40
Water		199	23.61	5.02	5.02
Control		180	11.11	1.66	1.66

LC_{30} 1.650 mg/egg (33.062 mg/kg)

LC_{50} 5.426 mg/egg (108.533 mg/kg)

LC_{90} 99.122 mg/egg (1982.446 mg/kg)

*Significantly different from solvent $P \leq 0.05$